Published in final edited form as:

Adv Exp Med Biol. 2014 ; 801: 49–56. doi:10.1007/978-1-4614-3209-8_7.

Primate Short-Wavelength Cones Share Molecular Markers with Rods

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Abstract

Macaca, *Callithrix jacchus* marmoset monkey, *Pan troglodytes* chim- panzee and human retinas were examined to define if short wavelength (S) cones share molecular markers with L&M cone or rod photoreceptors. S cones showed consistent differences in their immunohistochemical staining and expression levels compared to L&M cones for “rod” Arrestin1 (S-Antigen), “cone” Arrestin4, cone alpha transducin, and Calbindin. Our data verify a similar pattern of expression in these primate retinas and provide clues to the structural divergence of rods and S cones versus L&M cones, suggesting S cone retinal function is “intermediate” between them.

Keywords

L&M opsins; Monkey; Neural retina leucine zipper; Primate; Photoreceptor; S-antigen; S opsin; Visual arrestins

7.1 Introduction

The primate retina has one type of rod photoreceptor and three types of cone photoreceptors. In humans, rods outnumber cones 15–20:1 and form a single group that expresses photosensitive rhodopsin in their outer segments (OS), while 90 % of cone OS have

photosensitive pigments containing either long- (L or red) or medium-wavelength (M or green) selective opsin or 5–10 % contain short-wavelength (S or blue) selective opsin.

The cones expressing L or M opsin are remarkably similar in their anatomy and physiology, but the cones with S opsin have unique differences, reviewed in [1]. S opsin has a 42 % molecular homology to rod, L or M opsin and is an “intermediate” visual opsin [2]. Thus, specific reagents cannot distinguish L from M cones (L&M). However, S opsin specific reagents distinguish S from both L&M cones and rods. Morphologically, the S cone differs from the L&M cones in inner segment (IS) size and shape, smaller synaptic pedicle. Their cell body lies deeper from the external limiting membrane [3–5] and extracellular sheath around their OS that stains more heavily with peanut agglutinin [6, 7].

There are unexplained species differences within the primates for S cone distribution. In all primates, L&M cones are found throughout the retina in diminishing density from the foveal center. In macaque and marmoset, S cones are found at lower density throughout the fovea [8, 9]; while in human, S cones are lacking in the foveal center in both adults and fetuses [4–10]. In all Old World primates, S cones are spaced semi-regularly throughout the retina [11]. In New World squirrel monkey but not New World marmosets, S cones occur in small clumps [9].

Using specific antibodies for visual arrestins (Arr), transducins, and calbindin, we examined monkey, ape, and human retinas to define if S cones share molecular markers with rod or L&M cones photoreceptors.

7.2 Materials and Methods

7.2.1 Tissue

Adult Macaca monkey, Callithrix jacchus marmoset monkey, and Pan troglodytes chimpanzee eyes were obtained under authorized animal protocols from Tissue Programs at San Antonio, Emory, Washington, or Wisconsin Regional Primate Centers. Human eyes were obtained through the University of Washington, Willed Body Program. Eyes were enucleated, cornea and lens removed, and posterior globe immersion fixed in 4 % paraformaldehyde in 0.1 M phosphate buffer for 2–4 h. Selected areas of the retina were removed, cryo-protected, and serial frozen sections were cut at 12–20 μ m. Every tenth slide was stained with 1 % azure II-methylene blue in borax buffer pH 10.5 to identify retinal morphology and locate the fovea.

7.2.2 Immunohistochemical (IHC) Staining

Selected sections were double IHC labeled using a combination of mouse monoclonal (Mab) and rabbit polyclonal (Pab) antibodies. IHC labeling used standard methods [9]. Pab were generated to S cone opsin (JH455; 1/10,000, J. Nathans, Johns Hopkins), L&M cone opsin (1/5,000, J. Saari, Univ. Washington), human cone Arr4 (Luminaire Founders [hCARR-LUMIf]; 1/15,000), [12] S-antigen or “rod” Arr1 (C10C10; 1/10,000) [13], and rod alpha transducin (RTr; sc389; 1/2,000, Santa Cruz Biotechnology).

Mabs were generated to S cone opsin (OS2; 1/20,000, A. Szél, Semilweiss Univ.), Arr4 (7G6; 1/300, P. MacLeish, Morehouse Univ.), Arr1 (S-antigen, D9F2: 1/7,000, L.A. Donoso, Wills Eye Hospital), calbindin D28k (#C8666; 1/2000, Sigma-Aldrich), and alpha subunit of cone transducin (CTr; A1.1; 1/300, J. Hurley, Univ. Washington). For detection, the tissue was incubated in a mixture of 1/500 goat anti-rabbit IgG coupled to Alexa 488 and goat anti-mouse IgG coupled to Alexa 594 (Molecular Probes).

To detect differences in IHC labeling intensity within the photoreceptors, adjacent sections were incubated in primary antibodies diluted 2, 5, and 10 times normal working dilution. Sections were imaged either using a Zeiss LSM two-photon confocal microscope or a Nikon E1000 wide field digital microscope equipped with deconvolution software (Scientific Volume Imaging, The Netherlands). All images were processed in Adobe Photoshop CS3 for color balance, sharpness, and contrast.

7.3 Results

In all four primates, S cones showed a consistent difference in their IHC staining pattern compared to L&M cones, but some species differences were also noted.

7.3.1 Cone Arr4 (Pab LUMIf; Mab 7G6)

All cones heavily labeled throughout the IS, cell body, axonal Fiber of Henle (FH), and synaptic pedicle with both antibodies to Arr4 (Fig. 7.1a, b, c, d); rods were unlabeled. The S cone OS labeling intensity was much lighter than neighboring L&M OS or S cone cell body (Fig. 7.1a–d, arrows). At 5x dilution, S cone OS labeling was almost undetectable while L&M cone OS immunostained up to 10x dilution (data not shown).

7.3.2 Rod Arr1 (S-Antigen; Mab D9F2; Pab C10C10)

OS labeling pattern was similar for both antibodies to Arr1. L&M cone OS were negative for both antibodies, while the S cone OS labeled for both (Fig. 7.1e, f; arrow) at similar intensity as surrounding rod OS (Fig. 7.1e, f, R). In the marmoset and macaque fovea (Fig. 7.1g) and parafovea (Fig. 7.1h), OS2+Arr1 double IHC marked the distinctive sparse S cone OS (arrows) lying between the numerous unlabeled L&M cone OS. The S cone was heavily labeled from OS to pedicle. Some FH axons could be traced from Arr1+ S cone cell bodies (Fig. 7.1g, h; arrowheads). Heavy Arr1 IHC in rod OS (Fig. 7.1g, R; Fig. 7.1h, asterisk), IS, cell body (Fig. 7.1h, R), and axon can be seen on the foveal edge (Fig. 7.1g, right side) where rods are sparse. Mab D9F2 labeled the same regions of S cones and rods, but much less intensely (not shown).

7.3.3 Cone Alpha Transducin (CTr; Mab A1.1)

IHC labeling for CTr was similar to Arr4 for both cone types in all primates, but S cone OS varied in intensity (Fig. 7.1i) from light to dark. Most S cone OS were unstained at 5x dilution of A1.1.

7.3.4 Rod Transducin (RTr; sc389)

No labeling was detected in either L&M or S cones while rods were heavily labeled (data not shown).

7.3.5 Calbindin-D24k (CalB; Mab C8666)

In monkeys and chimps, all L&M and S cones labeled from IS to synaptic pedicle, with light to negligible labeling in the OS (Fig. 7.1j, k, arrow). In humans the OS were unstained and the S cone contained little CalB compared to surrounding L&M cones (Fig 7.1l, m, arrow).

7.4 Discussion

An earlier immuno-electron microscopy study showed that rod and S cone OS, but not L&M cone OS, in baboon retina are labeled with S-antigen, renamed “rod” Arr1 [14]. By contrast, Arr1 is expressed in all mouse rods and cones [15]. Later, a second visual arrestin, “cone” Arr4, was discovered that was highly expressed in all cones but no rods of many vertebrates [12, 16, 17]. Our data extend these earlier observations to several other primate retinas and verify a similar pattern of “intermediate” expression of both “rod” and “cone” visual Arr in S cones.

Close molecular ties exist during development between S cones and rods. The nuclear transcription factors, neural retina leucine zipper (NRL), and nuclear receptor subfamily 2, group E, member 3 (NR2E3), are essential for normal rod development. If one or both of these regulators are genetically altered, progenitors that should have a rod fate shift their genetic program to become S cones [18, 19]. Another similarity is that S cone inner retinal circuitry is more similar to that of rods than L&M cones [20, 21]. In central retina, two to five S cones converge onto a single “blue ON” bipolar cell and multiple rods converge onto a “rod” bipolar. In inner retina there is further convergence by blue bipolars onto a subset of ganglion cells. By contrast, a single L&M cone synapses onto a single “midget” ON and a single “midget” OFF bipolar. Each midget bipolar, in turn, synapses onto a single ganglion cell. Thus, this “midget” pathway is the basis of high visual acuity as well as red/green color vision, while the S cone system seems to be designed for chromatic sensitivity.

In all four primates, S cones showed a consistent difference in their IHC staining pattern and level of expression compared to L&M cones and rods. Both cone types labeled heavily for Cone Arr4 and CTr from IS to synaptic pedicle, but S cone OS were typically stained less intensely than L&M. “Rod” Arr1 did not label L&M primate cones, but S cones and rods were labeled heavily. In monkeys, the L&M cone cytoplasm, but not OS, was well labeled for CalB in both cone types, while in chimps and humans the S cone was lightly labeled. Rods were negative for CalB in all primates. Our results show that human, monkey, and ape S and L&M cones share Cone Arr4, CTr, and CalB expression. Only S cones share “rod” Arr1 expression with rods while RTr expression is confined to rods.

Why do S cones and rods share any molecular markers? It is possible that rod developmental signals are not turned off appropriately in the S cones, although Bumsted et al. found no coexpression of NRL or NR2E3 in primate cones [22]. Alternatively, perhaps the functional and structural similarities between the rhodopsin in rods and S opsin in cones recruit this

transduction shutoff molecule to maintain visual sensitivity with lower intensity light and to protect the rods and S cones from retinal degeneration.

Acknowledgments

Dr. Craft holds the Mary D. Allen Chair in Vision Research, Doheny Eye Institute. We thank Drs. Donoso, MacLeisch, Nathans, and Saari for generously providing anti- bodies. This work was supported, in part, by EY015851 (CMC), Kayser Award (AH), CORE grants EY01730 (UW) and EY03040 (DEI), and Research to Prevent Blindness. We gratefully acknowledge the assistance of the Willed Body Program and the Tissue Programs at University of Washington, University of Wisconsin Regional Primate Research Center (P51RR000167), San Antonio Primate Center (P51-RR13986), and Yerkes Regional Primate Center.

Abbreviations

Arr	Arrestin
Calb	Calbindin
CTr	Cone Transducin
IHC	Immunohistochemistry
S	Short wavelength
M	Medium wavelength
L	Long wavelength
RTr	Rod Transducin
Mab	Monoclonal
Pab	Polyclonal
OS	Outer segment
NRL	Neural retina leucine zipper
NR2E3	Nuclear receptor subfamily 2, group E, member 3

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Fig. 7.1.

Sections of adult primate retina stained IHC for the designated antibodies. **a–d** Cone Arr4 labeling in human peripheral retina (**a, b**) shows S cone opsin (*red*) labeled by Pab *Sop* and both S and L&M cone cytoplasm labeled by Mab *7G6* to hARR4 (*green*). Staining in macaque central (**c, d**) retina is similar for Mab *OS2* and Pab hCARR-LUMIf (*L1F*). In both combinations, S cone outer segments (*OS*; *arrows*, **a–d**) are labeled more lightly than L&M *OS*. Scale in **b** for **a–d**. **e, f** Chimpanzee S cone *OS* (*arrows*) label for Arr1 at a similar intensity to surrounding rod *OS*. **g** Marmoset foveal edge shows S cone *OS* labeling by *OS2* (*red*) and intense labeling (*green*) by Pab C10C10 to Arr1 of S cones *OS* (*arrows*), IS, cell body (*N*) and synaptic axon or fiber of Henle (*FH*). The axons angle away from the foveal center to the left. A few Arr1 + rod *OS* (*R*) are present on the edge of the fovea. **h** Macaque cones (*C*) and rods (*R*) both labeled for Arr1 from *OS* to cell body (*C, R*) to *FH* (*arrowhead*) by Pab C10C10 (*C10*). S cone *OS* (*arrow*) labeled similar to adjacent rod *OS* (*arrowhead*). **i**. Both L&M and S cone *OS* (*green*) label heavily for cone transducin (CTr) (*red*) with Mab *A1.1*. The cytoplasm from IS to pedicle heavily labeled in both (not shown). The S cone *OS* varies in CTr intensity, with some *OS* clearly double labeled (*arrowhead*) and others with lighter CTr labeling (*arrow*). **j, k** Macaque L&M cones labeled for calbindin with Mab *CalB* used at 2x dilution from IS to pedicle, but S cones only lightly labeled. Cone *OS* labeled lightly. **l, m** Human L&M cones label from IS throughout the rest of the cytoplasm for Mab *CalB* used at 2x dilution, but S cones show little labeling. Neither type of *OS* contains detectable labeling. Retinal pigment epithelium (*PE*) indicates inherent auto-fluorescence in this layer. Scale in **j** for **j–m**